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**Effect of Vibration Frequency and Acceleration
Magnitude of Chicken Embryos
on Viability and Development
Phase I**

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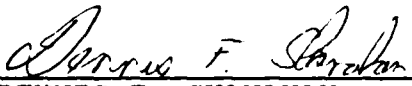
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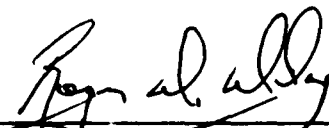
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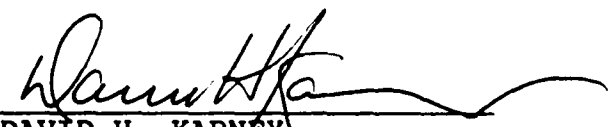
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Preface

Vibration exposure standards for pregnant women and their developing fetuses have not been established. Clearly, this is a highly complex issue that cannot be elucidated in a single study or even series of studies due to the extreme lack of data on the subject. To clarify the issues will require epidemiologic studies as well as basic scientific studies to explore the feasibility of developing an avian model to study the relationship of vibration to a developing embryo. The first step is to identify if chick development is inhibited by vibration at certain frequencies and amplitudes. If this pilot project is successful, it will be followed by a more thorough study to determine threshold vibration frequencies and amplitudes inhibiting chick development. Knowledge gained from these studies then can be applied toward the development of a basic mammalian model, eventually leading to a primate model. Ultimately, this line of research should provide scientifically valid guidelines to medical administrators who are tasked with setting the health and safety standards for the aviation community.

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Contents

	Page
Contents.....	1
Introduction.....	3
Methods.....	5
Materials.....	7
Results.....	7
Discussion.....	8
Conclusions.....	9
References.....	10
Appendix A. Manufacturer's list.....	12

List of tables

Table

1. Comparative embryology.....	6
2. Results.....	8

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Introduction

From December 1977 through August 1989, there were 93 women U.S. Army pilots grounded for pregnancy in accordance with AR 40-501, chapter 4-13b(1). The number of pregnant pilots per year has been increasing, with 23 of these suspensions from January through August 1989 (UES, 1989). Many pregnant Army helicopter pilots, being unaware of any potential hazard associated with vibration exposure, continue to fly during their first trimester, not reporting their pregnancy to their flight surgeon until after the 4th month to avoid over 6 months of medical suspension and administrative action. Thus, the true exposure rate of pregnant women to helicopter flight is unknown.

Air Force and Navy pilots are allowed to fly to 24 weeks with restrictions of below 10,000 feet, dual status, nonejection seat aircraft, and on a voluntary basis. Recently, women have called the Army's regulation grounding women when pregnancy is diagnosed discriminatory, as there has been no advisement of potential hazard issued (USAAMC, 1989; ODCSPER, 1989). One potential hazard is vibration exposure.

Currently, information about the effect of whole-body vibration (WBV) on the developing embryo is limited to a few animal studies and broad epidemiologic studies. Appropriate guidelines are dependent on development of valid laboratory and epidemiologic databases.

Varied epidemiologic approaches to the effect of vibration have been inconclusive. In one study comparing premature deliveries and low birth weight infants with case matched controls, there was no significant difference in noise and vibration exposure (Hartikainen-Sorri et al., 1988). Another occupational health study reported increased ratios for stillbirth for physical effort and vibration (McDonald et al., 1988). As stated in a 1982 report by the World Health Organization (WHO), a Hungarian report indicated changes in the pelvic organs and lumbar spine in females exposed to low frequency WBV: "It was concluded that methodological problems concerned with the measurement of dose and with different biological effects in the female need to be overcome before valid recommendations can be made." Compounding the difficulties of establishing the incidence of spontaneous abortion among women Army pilots as compared to other women Army officers is the high rate of spontaneous abortion prior to detection of pregnancy. One study reported "22% of all pregnancies detected by assay for HCG [human chorionic gonadotropin] failed to survive to the stage of being recognized clinically" (Wilcox et al., 1988).

A survey of animal studies in the literature indicated a possible harmful effect of vibration upon embryologic development. Two studies utilized the mouse as the animal model. In the first study, an acceleration of 5.6 gx at a vibration frequency of 20 Hz (mouse visceral resonance frequency) for 10 minutes at 4.5 days gestation (implantation stage) produced a 10.2 percent deformation rate compared to 1.6 percent in the control group. Birth weight of the offspring also was decreased. Of the three frequencies tested (5, 10, and 20 Hz), 20 Hz was the most damaging. Implantation stage was felt to be a vulnerable period for the embryo because of "the delicate balance of the synergistic action of estrogen and progesterone on the endometrium of the uterus for proper attachment of the embryo" (Bantle, 1971).

In the second study, exposure to 3.5 g (rms) at 45 Hz for 4 hours resulted in 40.9 percent resorption rate with 4 hours exposure, 22.2 percent with 8 hours exposure, and 5.6 percent rate for the controls. Hematomas were present in 5.9 percent of those receiving 4-hour exposures and 22 percent of those receiving 8 hours exposure, while hematomas were present in 4 percent of the control group. No teratologic effect was evident. It was felt probable [that] the stress in the female animals produced a constriction of blood vessels that impaired the placental function (Briese, Fanghanle, and Gasow, 1984).

Other studies used avian models. Chick embryos vibrated at 5 Hz and 5 mm amplitude (0.25 g) had decreased oxygen uptake on the 5th to 8th days of incubation. This corresponds to the rate of allantois development as it expands from a small area of dense network of blood vessels on the 5th day to covering the entire inner shell by the 9th day. The allantois takes over the oxygen uptake and carbon dioxide elimination function from the vitelline circulation (Lizurek, 1973). Japanese quail eggs exposed to vibration of 5, 10, 20, 30, 50, 80, and 100 Hz prior to incubation showed increased mortality, with most effect at 30 Hz (48.50 percent mortality as compared to 10.87 percent control mortality). Considering mortality in the 0-7 day period, 10, 20 and 30 Hz had mortality rates of 7.82, 10.73, and 10.22 in one trial with 1.59 percent for the control. Mortality rates at 20 and 30 Hz were 6.39 and 9.17 percent in the second trial with 3.90 percent for the control. In the first 7 days in all trials, 50, 80, and 100 Hz exposure caused 3.9 percent or less mortality (Sabo, Boda, and Peter, 1982).

One difficulty encountered in comparing the mammalian studies to humans is the maternal anxiety factor. It can be reasoned since pilots have chosen this profession, anxiety level would be expected to be minimal as compared to untrained animals exposed to vibration environments. The mechanical effect of vibration directly upon the embryo and its gas exchange structures is of

prime interest. Certainly, no direct comparison can be made between the effects of vibration on avian embryologic development and human development, but exposure guidelines can be developed as a base for higher animal studies, with trained primates of a size close to human as the ideal choice. In order to provide a basis for further development of exposure criteria given the broad range of vibration frequencies and intensities, it is preferable to work with an animal model with short development periods that is easily managed and has no maternal anxiety factors. The chick embryo meets these criteria.

The embryologic periods chosen for study were from development of the primitive streak and embryonic vessels at 20 hours of incubation through appearance of the beak on the 9th day. Vibration during this period exposes the developing organ systems and the beak and extremities, common sites of chick malformations, to the possibility of teratologies. Human pregnancy is easily diagnosed with common laboratory tests at the primitive streak period; therefore, the grounding regulation in question covers this period through the remainder of the gestation. The egg is laid about 24 to 27 hours after fertilization when the blastoderm has differentiated into two layers in a process called gastrulation. The bilaminar germ disc of the human forms by 7.5 days and on the 8th is partially imbedded in the endometrial stroma. The heart begins to beat by the 42nd hour of incubation for the chick and the 23rd day of human embryonic life. Limb formation starts at 62 to 64 hours of incubation in the chick and by the 5th week in the human. Feather germs appear on the 8th day of the chick incubation and hair buds by the 4th month of human development. The chick beak forms on the 6th through 10th days, with appearance of a beak on the 9th day. The human fetus develops a human looking face during the 3rd month (Langman, 1975; Stromberg, 1975; Lippincott, 1946).

Methods

One hundred chicken eggs were obtained for the project from Conagra Broiler Company. The eggs were divided into 4 trays holding 25 eggs each. The eggs were incubated in a Humidaire Model incubator* maintained at 99.5°F and 84-86° wet bulb for the first 18 days and 90-94° wet bulb for the last 4 days. Eggs automatically were turned hourly through the 18th day of incubation. The eggs were candled on the 4th, 10th, and 14th days of incubation. Exposures of the chick embryo to vibration for 15 minutes every 3 hours was chosen to give the embryonic stages exposures of the periodicity and length felt to be roughly equivalent to 3-hour exposures five times a week for the human embryo. This also was chosen for the practical need to avoid

* See manufacturers' list.

Table 1.
Comparative embryology.

Embryologic structures	Chick (beginning from incubation)	Human (from fertilization)
Bilaminar disc	Egg is laid (24 to 27 hours post fertilization)	7.5 days
Primitive streak	20 hours	15 to 16 days
Heart beat	42 hours	23 days
Limb formation	62-64 hours	5 weeks
Beak formation	6-10 days	----
Human looking face	---	3 months
Feather germs	8 days	---
Hair buds	---	4 months

cooling the eggs with more frequent or longer exposures. Temperature measurements were made with a temperature probe inserted into a raw egg. There was no measurable heat loss during 15 minutes of vibration in the holder under a heat lamp. Exposure frequencies were 1, 5, and 10 Hz. The 1-Hz frequency was selected because this proved to be the resonance frequency of the yolk within the egg white in an intact egg. (The most damaging frequency to mouse embryos in Bantle's study was mouse visceral resonance frequency.) This information was obtained by injecting an egg yolk with methylene blue through the shell and videotaping the candled egg as it was vibrated along its longitudinal axis while mounted with the long axis horizontal. Maximum yolk motion was seen in the region of 1 Hz when testing with swept sine frequency from 0.1 to 2.0 Hz. No motion was present below 0.6 or above 1.7 Hz; 5 Hz was selected because this frequency was proven in Lizurek's study to inhibit oxygen uptake by the chicken embryo. The next frequency increment for testing was arbitrarily selected as 10 Hz. Higher frequencies were not tested in the pilot project because the incubator trays would hold only 4 groups of 25. The control group was handled as the experimentals

except the vibration table was static during the times the control group was mounted on the table.

The 1-Hz group was accelerated at 1 g, and the 5-Hz and 10-Hz groups were vibrated at 3 g acceleration. These levels were the maximum levels allowed by the table and were selected in order to establish any possible effect of vibration upon the embryo.

Materials

The Materials Test System vibration table* used was a single-axis hydraulic table with vertical motion. Maximum acceleration at 1 Hz was 0.25 g. At 5 Hz, the table performed best at 3 g and below. The mount consisted of a wooden holder with lid lined with egg crate mattress and held on by four hinge pins. The wooden mount was attached to a metal sheet bolted to the table. A heat lamp was placed 2 feet above the table, ensuring no measurable heat loss from the egg during 15 minutes on the table.

The incubator was a Humidaire Model 21 self-turning incubator*. The four trays were lined with egg crate foam mattress material.

Results

In the control group, 17 chicks hatched on the 21st day and 4 on the 22nd day, giving an 84 percent hatch rate. During the 13th day of incubation, the incubator was inadvertently turned off for 22 hours, allowing the incubator to cool to 74°F before the problem was discovered and corrected. Despite this, the hatch rate was within the 95 percent confidence level of the Conagra hatch rates over 15 months (mean rate = 85.54; standard deviation = 1.73; n = 57). One embryo appeared to have died within the first 2 days, one at the 5-day stage, one at the 8-day stage, and one at 18 days.

In the 1-Hz group, 8 hatched at 21 days and 9 hatched at 22 days, giving a hatch rate of 68 percent. Breakage of eggs occurred in the 1-Hz group. Some breakage was known to occur because of slipping of the tray within the incubator as it automatically turned at the time the adjacent tray was removed for vibration exposure. Others apparently cracked during vibration. Three eggs cracked on the 2nd day of incubation, two eggs on the 3rd day, one egg on the 4th day, and one on the 7th day. The only cracked egg that hatched was the one that cracked on the 7th day. If cracked eggs are excluded, 16 of 18 eggs hatched for a rate of 89 percent. One egg showed no evidence of fertility, 4 embryos

appeared to have died at the 3-day stage, 2 at 14 days, and 1 at 18 days.

In the 5-Hz group, none hatched. One egg contained a small amount of blood. All others showed no development, rather, an amorphous sludge for the yolk.

In the 10-Hz group, three eggs hatched. One hatched on the 21st day and two on the 22nd day. Hatch rate was 12 percent. One egg appeared infertile, 16 eggs contained amorphous tissue, 2 had blood lines present, 1 embryo appeared to be 2 days old, 1 appeared 3 days old, and 1 died at 20 days development. There were two anomalies among the hatchlings. One was missing an eye and had an incompletely formed palpebral fissure in the other eye, and the other had an umbilicus that did not dry. This chick died at 3 days. Because of technical problems, the chicks became mixed and the groups from which these anomalies occurred could not be determined.

Table 2.

Results.

Group	1 Hz .25 g	1 Hz .25 g*	5 Hz 3 g	10 Hz 3 g	Controls
Hatched	17	16	0	3	21
Incubated	25	18	25	25	25
Percent hatched	54%	89%	0%	12%	84%

* 1 Hz eggs that did not crack

Discussion

The forces to which the chick embryos were exposed were far in excess of that allowed for human tolerance exposure in the ISO standard (ISO 2631, 1985). However, it has been established that severe vibration results in mortality to developing chick embryos in the early stages despite their fluid surrounding. In comparing the 5-Hz group to the 10-Hz group, it appears that the 5-Hz frequency is more lethal than the 10-Hz frequency at the same acceleration. It could be argued that excursion may be the determining factor, as the excursion is less at 10 g than at 5 g; however, this is contradicted by the relatively high hatch rate

of the 1-Hz group which had the greatest excursion. Movement of the "host," i.e., the yolk at resonance, appears to have little effect upon the embryo, though the technical problems of egg breakage prevent any comparison of the 1-Hz group to the controls. It should be noted that 0.25 g at 1 Hz is well within the fatigue-reduced discomfort boundary of ISO, whereas 3 g's at 5 Hz and at 10 Hz is six times the force allowed for human tolerance by ISO. However, as tolerance to vibration varies with the organism size, no direct comparison between chick embryos and human adults is possible.

This study has established that vibration of certain magnitudes and accelerations is lethal to developing chick embryos. It is hoped with further animal studies to gain more knowledge of the effects of vibration on embryo development.

The ultimate goal of continued laboratory and epidemiologic research of the effects of vibration on developing embryos is that a standard for exposure of pregnant women will be developed with the same utility as the current ISO standard of vibration for the general population. This project is an early step along the path of further knowledge. It is clear no simple avian study can be generalized to policies concerning humans, but the results of this project can guide further studies.

Conclusions

Vibration of sufficient amplitude and appropriate frequency is lethal to developing chick embryos without respect to the lack of relative motion of the yolk within the egg. Further studies are needed to define the frequencies and acceleration forces that are responsible for destruction of the embryo in its early stages. This pilot project will be followed by a more thorough study to determine threshold vibration frequencies and amplitudes inhibiting chick development.

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Appendix A.

Manufacturers' list.

Humidaire Incubator Company
217 West Wayne Street
New Madison, OH 45346

MTS Systems Corporation
Box 24012
Minneapolis, MN 55424

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